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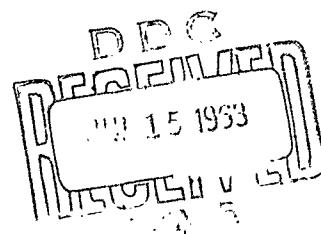
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TECHNICAL MANUSCRIPT 67

TRANSDUCTION IN A LYSOGENIC STRAIN OF BACILLUS ANTHRACIS USING HOMOLOGOUS PHAGE

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TRANSDUCTION IN A LYSOGENIC STRAIN OF BACILLUS ANTHRACIS
USING HOMOLOGOUS PHAGE

The work reported here was performed under Project 4892-02-034, "BW Agent Process Research," Task -04, "Stock Culture." The expenditure order was 2029. This material was originally submitted as manuscript 5124.

Albert John Rosenwald

Ira Cecil Felkner

Ralph Ernest Lincoln

Process Development Division
DIRECTOR OF DEVELOPMENT

Project 1C022301A074

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ABSTRACT

A phage common to both donor and recipient was used in transductions of streptomycin resistance and streptomycin dependence in *B. anthracis*. The rates of transduction were approximately 10^{-7} per colony-forming unit of the recipient population.

I. INTRODUCTION

The occurrence of transparent plaque-like changes in aged cultures of B. anthracis was mentioned by Ivanovics and Lantos,¹ who observed that most authors had failed to demonstrate the presence of any transferrable agent in them. We observed similar plaque-like formations in old cultures of B. anthracis. Although these changes suggested the presence of bacteriophage, preliminary studies based on phage propagation and on electron microscopy were unsuccessful in proving the case. However, sterile filtrates derived from strains carrying a drug marker were found capable of transferring that marker to a recipient strain. The transfer was insensitive to deoxyribonuclease and was judged to be a transduction.

In addition to the transduction described, the present work indicates a potentially useful method for testing cultures of B. anthracis, and perhaps other organisms, in which the presence of phage is known or suspected but which other generally used methods have failed to demonstrate.

II. MATERIALS AND METHODS

Bacillus anthracis, strain Vollum, which is both virulent and sporegenic, was used in these experiments. The streptomycin-resistant and streptomycin-dependent strains were obtained as single-colony isolates from the original strain after ultraviolet irradiation. These isolates were tested by streaking and replica-plating on agar medium with and without streptomycin and each appeared to be pure for its specific drug marker.

The medium used was Difco brain-heart infusion (BHI), as liquid (25 milliliters per 250-milliliter flask), or solid (one per cent agar). Streptomycin sulfate (Consolidated Midland Corporation) was used at a level of one milligram per milliliter where indicated. Cultures were incubated at 37°C unless otherwise specified.

The presence of phage in the Vollum strain was first indicated by the appearance of spontaneous plaques on old cultures on agar. Following this observation, cultures of the Vollum strain were induced with ultraviolet light by irradiating 10 milliliters of culture in an open Petri dish (nine centimeters in diameter) for 40 seconds at a distance of nine inches from a standard 15-watt germicidal lamp. The culture was agitated during irradiation. The irradiated culture was put into a 250-milliliter flask and incubated on a shaker for 1.5 hours at 37°C. No appreciable amount of lysis was observed.

The cells then were removed from the culture by centrifugation at 2000 revolutions per minute for 30 minutes in an angle-head centrifuge and the centrifugate sterilized by passage through an ultra-fine fritted glass filter. Plaque assays were made of a number of such filtrates on lawns of Vollum, Sterne, 30R, and HBA strains. Plaques were not observed at any dilution in these assays, but spot tests of the filtrates were faintly positive on strains Sterne, 30R, and HBA, and negative on Vollum.

An attempt was made to cure the Vollum strain of its lysogeny and render it sensitive by serially passing it in BHI to which oleic (15 to 20 micrograms per milliliter)³ and oxalic acids (M/125)³ had been added. Spot tests on this treated culture were faintly positive in early transfers but became increasingly clear by the twelfth transfer. At this time, attempts to use this treated culture to assay for plaque-forming units were unsuccessful and culture treatment was discontinued.

To perform the transduction of streptomycin resistance, a sterile filtrate of a 16-hour streptomycin-resistant culture was prepared using the induction procedure already described. Fresh filtrates were prepared for every experiment and used immediately. To rule out genetic transfer by transformation, equal volumes of filtrate and a solution of 20 micrograms per milliliter of deoxyribonuclease (Worthington Biochemical Corporation) were mixed and incubated at 37°C for 30 minutes. Experiments by I.C. Felkner had shown deoxyribonuclease to be effective in preventing transformation of streptomycin resistance under these conditions. The treated filtrate then was added (10 per cent v/v) to a fresh, three-hour subculture of streptomycin-sensitive Vollum. After static incubation for 20 to 30 minutes at room temperature, one-milliliter quantities of the treated culture (about 10⁸ colony-forming-units) were impinged onto Millipore filters (type HA, 0.45 micron, 47-millimeter diameter) which were incubated for four hours at 37°C on BHI agar without streptomycin to allow expression of the resistance characteristic. The filters then were transferred to BHI agar with the drug incorporated. Controls were prepared in equal quantity on filters using the recipient culture except that the culture was not treated with filtrate. Transductions of streptomycin dependence were done in the same way with the exception that the donor culture was grown for 16 hours in BHI broth containing streptomycin; then the cells were removed by centrifugation, resuspended in an equal volume of broth with no streptomycin, and shaken at 37°C for an additional 24 hours. Following the second incubation period, the culture was induced with ultraviolet light and a filtrate was prepared as previously described.

Because we have not found a strain of B. anthracis suitable for assay of plaque-forming units, transduction efficiency is reported as transductants per colony-forming unit (cfu) of recipient population. Because of the chaining characteristics of the organism, a colony-forming unit should be regarded as representing an approximate number of cells.

III. RESULTS

Table I shows transduction efficiency of the determinant for streptomycin resistance. These figures reflect the total number of colonies observed on the Millipore filters in each experiment and are corrected for spontaneous mutants observed on control filters. The rate of spontaneous mutation observed in each experiment is also shown. The average rate of transduction of streptomycin resistance was 2.9×10^{-7} transductants per cfu of the untreated recipient population. The average rate of spontaneous mutation to resistance in the recipient population was 0.4×10^{-7} per cfu. The average rate of transduction in the cultures treated with oleic and oxalic acids was 3.7×10^{-7} per cfu. Spontaneous mutants were not observed in acid-treated controls.

Table I also shows transduction efficiency of the determinant for streptomycin dependence. The average rate of transduction of streptomycin dependence was 1.3×10^{-7} transductants per cfu. The average rate of spontaneous mutation to dependence in the recipient population was 0.17×10^{-7} per cfu.

When transductant colonies were replica-plated onto BHI and then onto BHI plus streptomycin they were found, regardless of the donor type, to represent an assortment of resistant, dependent, and abortive types, as well as colony types that were nonreplicable on solid medium with or without streptomycin. Average percentages of the various colony types occurring among the transductants from the streptomycin-resistant and the streptomycin-dependent donors are shown in Table I.

A significant number of the colonies observed on the Millipore filters did not correspond to the donor type; that is, some dependent types were found when the donor was resistant, and vice versa. The rate of occurrence of these aberrant types with the dependent donor (11.7 per cent) was somewhat less than the rate of spontaneous mutation (13.3 per cent), but, with the resistant donor, the aberrant types seemed to occur with a frequency greater than that of spontaneous mutation (37 vs 14 per cent).

When the determinant for streptomycin resistance was donated, about 20 per cent of the affected cells seemed, at least phenotypically, to have accepted the marker functionally as it existed in the donor; that is, they were resistant to streptomycin and grew equally well in its presence or absence. The remainder (minus aberrant types) appeared to be defective recipients. Some produced no colony on the filters but, upon replication, produced a colony on medium without streptomycin but not on medium containing the drug, suggesting abortive transduction. Others formed a visible colony on the filters but were not replicable on medium with or without streptomycin.

TABLE I. RATE OF TRANSDUCTION OF STREPTOMYCIN RESISTANCE AND STREPTOMYCIN DEPENDENCE IN BACILLUS ANTHRACIS USING HOMOLOGOUS PHAGE

Donor Type	Transduction	Spontaneous	Per Cent Transductant Types ^a /		
	Rate, $10^{-7}/\text{cfu}$	Mutation Rate, $10^{-7}/\text{cfu}$	Resistant	Dependent	Defective
Streptomycin	8.9	1.2			
Resistant	0.65	0.04			
(Untreated	1.0	0.19			
recipient)	1.0	0.23			
Average	2.9	0.4	20.5	37.0	42.5
Streptomycin	4.3	None detected			
Resistant	3.3	None detected			
(Acid-treated	3.6	None detected			
recipient) ^b /					
Average	3.7	0		Not determined	
Streptomycin	1.7	0.4			
Dependent	2.6	None detected			
(Untreated	0.1	0.03			
recipient)	0.7	0.19			
	1.3	0.23			
Average	1.3	0.17	11.7	65.4	22.6

a. Based on total replicable and non-replicable types.

b. Recipient culture serially transferred seven times in BHI plus oleic and oxalic acids.

When the determinant for streptomycin dependence was donated, at least two-thirds of the affected cells appeared to accept the marker for dependence and the remainder appeared to be defective recipients of that same marker. Of those that accepted the marker in a functional state, not all seemed to have accepted it in the same way. Using colony size as an indication of growth capability, colonies were observed that were dependent on different levels of drug, the larger ones apparently finding the provided level near optimum for growth and the smaller ones finding this same level unsuitable. Other dependent colonies, upon replication, appeared to be a mixture of dependent and resistant cells, and grew heavily in the presence of streptomycin but only faintly in its absence.

IV. DISCUSSION

Transduction of streptomycin resistance and streptomycin dependence by phage present in the Vollum strain of *B. anthracis* is described. In addition to the transfer of genetic characteristics, these transductions were used to demonstrate the presence of phage in a suspected lysogenic culture. The transductions reported are not comparable with other, more classical, transductions, being somewhat lower in efficiency and lacking supplementary verification by electron micrography and neutralization by a specific immune serum. They are based on the mathematical probability that a mechanism of genetic transfer was in operation and on other evidence that indicated that the transfer was due to transduction by a phage already present in the organism.

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